ORIGINAL ARTICLE

An Inactivated Cell-Culture Vaccine against Yellow Fever

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ABSTRACT

BACKGROUND

Yellow fever is a lethal viral hemorrhagic fever occurring in Africa and South America. A highly effective live vaccine (17D) is widely used for travelers to and residents of areas in which yellow fever is endemic, but the vaccine can cause serious adverse events, including viscerotropic disease, which is associated with a high rate of death. A safer, nonreplicating vaccine is needed.

METHODS

In a double-blind, placebo-controlled, dose-escalation, phase 1 study of 60 healthy subjects between 18 and 49 years of age, we investigated the safety and immunogenicity of XRX-001 purified whole-virus, β-propiolactone–inactivated yellow fever vaccine produced in Vero cell cultures and adsorbed to aluminum hydroxide (alum) adjuvant. On two visits 21 days apart, subjects received intramuscular injections of vaccine that contained 0.48 μg or 4.8 μg of antigen. Levels of neutralizing antibodies were measured at baseline and on days 21, 31, and 42.

RESULTS

The vaccine induced the development of neutralizing antibodies in 100% of subjects receiving 4.8 μg of antigen in each injection and in 88% of subjects receiving 0.48 μg of antigen in each injection. Antibody levels increased by day 10 after the second injection, at which time levels were significantly higher with the 4.8-μg formulation than with the 0.48-μg formulation (geometric mean titer, 146 vs. 39; P<0.001). Three adverse events occurred at a higher incidence in the two vaccine groups than in the placebo group: mild pain, tenderness, and (much less frequently) itching at the injection site. One case of urticaria was observed on day 3 after the second dose of 4.8 μg of vaccine.

CONCLUSIONS

A two-dose regimen of the XRX-001 vaccine, containing inactivated yellow fever antigen with an alum adjuvant, induced neutralizing antibodies in a high percentage of subjects. XRX-001 has the potential to be a safer alternative to live attenuated 17D vaccine. (Funded by Xcellerex; ClinicalTrials.gov number, NCT00995865.)

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Yellow fever, a lethal, mosquito-borne flavivirus disease, occurs in tropical South America and Africa. A live attenuated vaccine (17D) developed in 1936 is widely used, with approximately 20 million doses distributed annually. Although remarkably immunogenic, the 17D vaccine may cause serious viscerotropic and neurotropic adverse events and anaphylaxis. Viscerotropic disease is a fulminant 17D virus infection of the liver and visceral organs resembling naturally acquired yellow fever. Neurotropic disease typically follows invasion of the brain by the replicating vaccine virus.

Fifty-nine cases of viscerotropic disease (38 of which [64%] were fatal) have been reported since 1973.[1] In the United States each year, approximately 400,000 doses of 17D vaccine are administered, with up to two cases of viscerotropic disease reported. The rates of reported cases are 0.4 per 100,000 population for viscerotropic disease, 0.8 per 100,000 population for neurotropic disease, and 1.8 per 100,000 population for anaphylaxis.[2]

The use of a vaccine incapable of replicating in the host should circumvent serious adverse events and could be used in persons with precautions and contraindications against 17D vaccination: the elderly, immunosuppressed or thymectomized patients, infants under 9 months of age, and pregnant or nursing women. Use of cell-culture vaccines should reduce the risk of allergic reaction, since they do not contain the common allergens — egg proteins and gelatin — present in the 17D vaccine. The rationale for developing a new vaccine against yellow fever has been reviewed recently.[3]

XRX-001 is an investigational yellow fever vaccine manufactured by Xcellerex from the 17D strain grown in Vero cells. Virus is purified from culture fluid, inactivated with β-propiolactone, adsorbed to 0.2% aluminum hydroxide (alum), and formulated with stabilizers.[4] The vaccine has proved to be highly immunogenic in mice, hamsters, and monkeys and has provided protection against yellow fever virus on challenge testing.[4] We performed the first clinical study of XRX-001.

METHODS

STUDY DESIGN AND OBJECTIVES

From January through April 2010, we enrolled 60 men and women 18 to 49 years of age in a controlled clinical trial at one site in the United States. Subjects were recruited from the greater Kansas City, Kansas, community with the use of advertisements approved by the institutional review board and from lists of persons previously indicating a desire to participate in clinical trials. Xcellerex sponsored and designed the study; data were collected by the investigators and analyzed by Veristat; the manuscript was written principally by the first author. All the authors had full access to the data, contributed to the writing of the manuscript, made the decision to submit the manuscript for publication, and vouch for the completeness and accuracy of the data and data analysis presented.

The study had institutional-review-board approval and was conducted in accordance with the protocol (available with the full text of this article at NEJM.org) and with Good Clinical Practice guidelines and the provisions of the Declaration of Helsinki. The study sponsor, investigators, and subjects were unaware of the study-group assignments.

The objective was to determine the safety, adverse event profile, and immunogenicity of one injection and two injections of the XRX-001 vaccine at two dose levels. The coprimary immunogenicity outcomes were the proportion of subjects with seroconversion and the geometric mean titer of neutralizing antibodies. Secondary outcomes were the distribution of titers and duration of antibody response. Safety was assessed on the basis of local and systemic reactions and clinical laboratory abnormalities.

VACCINE

The vaccine consists of yellow fever 17D virus grown in Vero cells on microcarrier beads in a stirred-tank bioreactor containing serum-free medium. The supernatant containing virus is filtered, treated with nuclease to digest host-cell DNA, ultrafiltered and dialyzed, inactivated with β-propiolactone, further purified by means of Cellufine sulfate chromatography, adjusted to obtain the required potency, and adsorbed to 0.2% alum.[4] The stabilizing buffer contains TRIS hydrochloric acid, sodium chloride, magnesium chloride, glutamic acid, mannitol, and trimethylamine-*N*-oxide, with a pH of 7.5.

In this study, the low-dose (0.48-μg) vaccine formulation corresponded to 7.3 $\log_{10}$ virus equivalents per 0.5-ml dose, which is the inactivated-

antigen equivalent of live-virus plaque-forming units as measured by monoclonal enzyme-linked immunosorbent assay.[4] The high-dose (4.8-μg) formulation was equivalent to 8.4 $\log_{10}$ virus equivalents per 0.5 ml.

STUDY-GROUP ASSIGNMENTS AND FOLLOW-UP

Eligible subjects were clinically healthy; had normal clinical laboratory tests; had not previously been vaccinated against yellow fever, Japanese encephalitis, or tick-borne encephalitis; and did not have a history of military service overseas or travel in the tropics. All patients provided written informed consent before enrollment. Serum samples were obtained before vaccination (day 0) to check the yellow fever serologic status at baseline. Women were required to have a negative pregnancy test at the screening visit and before each vaccination.

Subjects were recruited in two cohorts according to a dose-escalating, double-blind design. Each cohort consisted of 30 subjects, 24 of whom received XRX-001 and 6 of whom received placebo (0.9% saline). In each of the two cohorts, subjects were randomly assigned to receive two 0.5-ml intramuscular injections, at an interval of 21 days, of XRX-001 at the low dose, XRX-001 at the high dose, or placebo. Blood samples for antibody testing were obtained on day 0, day 21 (before the second injection), day 31, and day 42.

With a list of possible adverse events and digital thermometers provided by the investigator, subjects recorded daily oral temperature and injection-site and systemic adverse events through day 42, using a grading scale based on interference with normal daily activities. On days 3, 10, 21, 24, 31, and 42, subjects returned to the clinic for inspection of the injection site and assessment of adverse events. Blood specimens for clinical laboratory evaluation were obtained at the screening visit and on days 10, 31, and 42.

ASSAYS

Immunogenicity was assessed by means of a serum-dilution, constant-virus plaque-reduction neutralization test (PRNT) against the 17D virus (Focus Diagnostics) that had been formally qualified and shown to be precise and linear. The end point was the highest serum dilution inhibiting 50% or more of the plaques, hereafter called the $PRNT_{50}$ titer. Seropositivity was defined as a $PRNT_{50}$ titer of 10 or greater.[5,6] Seroconversion was defined as an increase in the $PRNT_{50}$ titer from baseline by a factor of 4 or more. Assuming a baseline titer of 5 (one dilution below the starting dilution), seroconversion was considered to be achieved at a titer of 20 or greater in a postvaccination sample.

STATISTICAL ANALYSIS

Statistical analyses were two-tailed and assessed at the 5% significance level. Rates of seropositivity and seroconversion were compared between study groups with the use of Fisher's exact test and binomial 95% confidence intervals. The adjusted (least-square) geometric mean titer was compared across the study groups by means of analysis of variance. The Bonferroni correction was used for pairwise comparisons of the two vaccine doses with placebo, with a separate correction for the primary analyses of the rate of seroconversion and the geometric mean titer. We used the log-rank test to measure differences between study groups with respect to the cumulative reverse distribution of titers. Immunogenicity was assessed for the per-protocol population: all subjects who were seronegative at baseline, received the assigned study vaccine or placebo on day 0 and on day 21, underwent antibody testing of specimens obtained on day 0 and day 42, and had no major deviations from the protocol.

In the safety population (all subjects who received at least one dose of vaccine), we compared the proportions of subjects with adverse events across the two vaccine groups and the placebo group, using Fisher's exact test.

RESULTS

STUDY POPULATION

Fifty-seven subjects made up the per-protocol population; two subjects in the high-dose vaccine group were excluded because of the presence of antibody against yellow fever at baseline, and one subject in the placebo group was lost to follow up. The two seropositive subjects were most likely immunized during military service but were unaware that they had received the yellow fever vaccine.

All 60 subjects were included in the safety population. The mean age was 32 years; 35 of the 60 subjects (58%) were male, 41 (68%) were white, and 19 (32%) were black. Demographic and baseline characteristics were similar across all three study groups, with no significant differences.

Table 1. Incidence of Adverse Events Occurring in Two or More Subjects in Any of the Study Groups, According to Study Group.

Adverse Event*	High-Dose Vaccine (N=24)	Low-Dose Vaccine (N=24)	Placebo (N=12)
		number of subjects (percent)	
Days 0–21 (day of 1st injection–21 days after 1st injection)			
Injection-site symptoms			
Pain	13 (54)	13 (54)	2 (17)
Tenderness	15 (62)	19 (79)†	4 (33)
Redness	22 (92)	21 (88)	10 (83)
Swelling	22 (92)	21 (88)	10 (83)
Itching	3 (12)	1 (4)	0
Systemic events			
Diarrhea	0‡	4 (17)	3 (25)
Malaise	4 (17)	2 (8)	3 (25)
Headache	2 (8)§	8 (33)‡	7 (58)
Tiredness	7 (29)	2 (8)‡	5 (42)
Nausea	3 (12)	0	1 (8)
Days 22–42 (21 days after 2nd injection)			
Injection-site symptoms			
Pain	6 (25)	8 (33)	0
Tenderness	8 (33)	10 (42)‡	0
Redness	12 (50)	16 (67)	7 (58)
Swelling	12 (50)	16 (67)	7 (58)
Systemic events			
Vomiting	1 (4)	2 (8)	3 (25)
Malaise	1 (4)¶	4 (17)	5 (42)
Headache	1 (4)¶	6 (25)	5 (42)
Muscle ache	0†	3 (12)	3 (25)
Feverishness	0†	3 (12)	3 (25)
Chills	0	2 (8)	1 (8)
Tiredness	2 (8)	4 (17)	4 (33)
Nausea	1 (4)	3 (12)	3 (25)
Drowsiness	1 (4)	5 (21)	3 (25)
Shortness of breath	0	2 (8)	0
Days 0–42			
All events			
Upper respiratory infection	2 (8)	1 (4)	1 (8)
Muscle strain	0	0	2 (17)
Increased white-cell count	1 (4)	4 (17)	2 (17)
Back pain	0	2 (8)	0

* Reports of events were solicited (on a list of possible events provided by the investigators), except for the systemic events for days 0–42, which were unsolicited.
† P=0.02 for the comparison with the placebo group by Fisher's exact test.
‡ P=0.03 for the comparison with the placebo group by Fisher's exact test.
§ P=0.002 for the comparison with the placebo group by Fisher's exact test.
¶ P=0.01 for the comparison with the placebo group by Fisher's exact test.

Table 2. Rates of Seropositivity and Seroconversion and Geometric Mean Titer of Antibody in the Per-Protocol Population, According to Study Group.*

Variable	High-Dose Vaccine (N=22)	Low-Dose Vaccine (N=24)	Placebo (N=11)
Day 21 (21 days after 1st injection)			
Seropositivity — % (95% CI)	46 (24–68)	13 (3–32)	0 (0–29)
Seroconversion — % (95% CI)	32 (14–55)	13 (3–32)	0 (0–29)
Geometric mean titer (95% CI)	10 (7–15)	6 (5–8)	5 (5–5)
Day 31 (10 days after 2nd injection)			
Seropositivity — % (95% CI)	100 (85–100)	88 (68–97)	0 (0–29)
Seroconversion — % (95% CI)	100 (85–100)	75 (53–90)	0 (0–29)
Geometric mean titer (95% CI)	146 (104–203)	39 (22–69)	5 (5–5)
Day 42 (21 days after 2nd injection)			
Seropositivity — % (95% CI)	100 (85–100)	88 (68–97)	0 (0–29)
Seroconversion — % (95% CI)	100 (85–100)	71 (49–87)	0 (0–29)
Geometric mean titer (95% CI)	113 (81–159)	30 (18–49)	5 (5–5)

* Seropositivity was defined as a yellow fever–neutralizing antibody titer of 10.0 or more. Seroconversion was defined as an increase by a factor of 4 or more in the baseline titer (i.e., a titer ≥20.0, assuming a baseline titer of 5, one dilution below the starting dilution). A titer of 5.0 was also assumed to be the baseline titer in the calculation of the geometric mean titer. CI denotes confidence interval.

SAFETY

There were no serious adverse events. Table 1 lists the incidences of solicited adverse events during the 21-day period after each vaccine injection and of unsolicited adverse events throughout the 42-day double-blind treatment period. Local pain and tenderness were more frequent in the two vaccine groups than in the placebo group, but the frequencies did not differ according to dose. Local reactions were less frequent after the second vaccination than after the initial vaccination and were generally mild; moderate reactions were infrequent, and no severe reactions were reported. Tenderness after the first vaccination was more frequent in the low-dose vaccine group than in the placebo group (P=0.02). There were no febrile reactions. Solicited systemic adverse events were more common, in some cases significantly so, in the placebo group than in either vaccine group (Table 1).

A possible safety signal was one case of mild urticaria in the high-dose vaccine group, 3 days after the second injection. Unsolicited events occurring in two or more subjects in any group included an increased white-cell count, upper respiratory infection, muscle strain, and back pain (Table 1); the incidences did not differ significantly between the two vaccine groups. Hematologic and biochemical abnormalities were infrequent, mild, and showed no significant relationship to study group or dose.

IMMUNE RESPONSE

A single injection of XRX-001 elicited the development of neutralizing antibodies in 46% and 13% of subjects in the high-dose vaccine group and the low-dose vaccine group, respectively. Two injections were required for effective immunization. The antibody response after the second injection was rapid, with antibodies present in 100% of subjects in the high-dose group and 88% of subjects in the low-dose group within 10 days (Table 2).

Seropositivity and seroconversion occurred in more subjects in the high-dose group than in the low-dose group on days 21, 31, and 42 (Table 2), with significantly higher rates of seropositivity on day 21 (P=0.02) and rates of seroconversion on day 31 (P=0.02) and day 42 (P=0.01). Similarly, the geometric mean titers on day 31 and day 42 were significantly higher in the high-dose group than in the low-dose group (146 vs. 39 on day 31 and 113 vs. 30 on day 42, P<0.001 for both comparisons).

The neutralizing antibody titers measured by means of $PRNT_{50}$ in each subject (as well as the

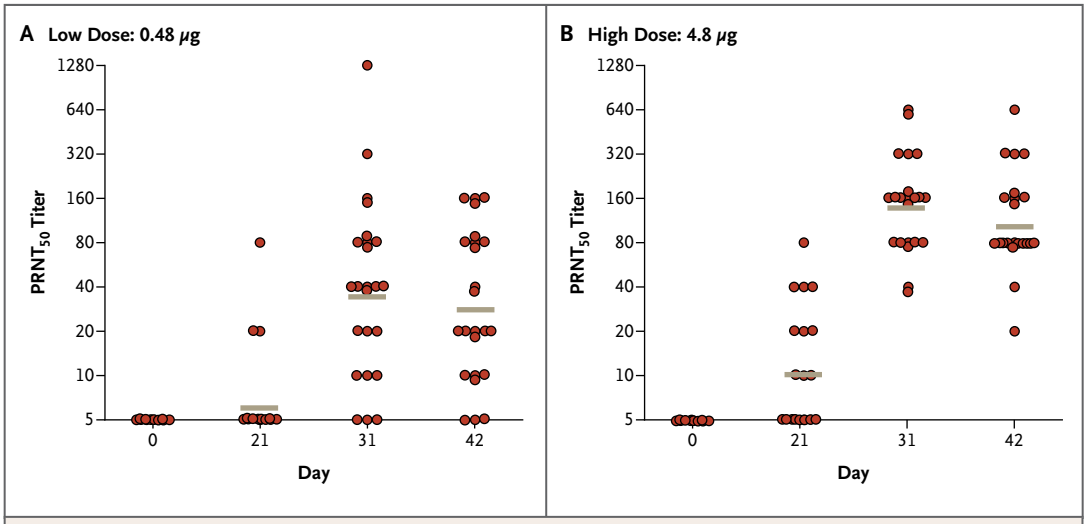

Figure 1. Plaque-Reduction Neutralization Test (PRNT) Titers of Neutralizing Antibody through Day 42 in the Two Vaccination Groups, According to Dose.

Panel A shows data for the low-dose vaccination group, and Panel B data for the high-dose vaccination group. The $PRNT_{50}$ titer is the highest serum dilution inhibiting 50% or more of the plaques. Values for each subject and the geometric mean titers (horizontal gray lines) are shown.

geometric mean titer at each time point) during the study period are shown in Figure 1, and the reverse cumulative distribution of titers is shown in Figure 2. The high-dose and low-dose vaccine groups differed significantly with regard to the distribution of titers ($P<0.001$) (Fig. 2). More than 95% of subjects in the high-dose group had neutralizing antibody titers of 20 or greater, and more than 90% had titers of 40 or more.

DISCUSSION

The XRX-001 vaccine was associated with modest local reactions, with no significant increase in the frequency of solicited systemic adverse events, as compared with placebo, and with few unsolicited adverse events. Pain and tenderness occurred more frequently in the two vaccine groups than in the placebo group, but the frequency did not differ significantly on the basis of the dose of antigen, suggesting that the alum or, less likely, one of the excipients (all of which are classified as substances generally regarded as safe) in the vaccine was the cause.

In hamsters and monkeys, a single injection of XRX-001 elicited antibody responses similar to those against the live 17D vaccine,[4] and a single injection would be an ideal regimen for travelers. In our clinical study, however, two injections of XRX-001 were necessary for effective immunization, as measured on the basis of the development of antibody titers. This finding could reflect the proportionally lower antigen dose per unit of body weight in humans, since a steep dose–response curve was observed in preclinical studies.[4] However, increasing the dose of a similar vaccine against another flavivirus infection, Japanese encephalitis, from 6 to 12 μg failed to permit the conversion of a two-dose regimen to a single-dose regimen.[7] The high antigen dose used in the current study is within the dose range for other approved flavivirus vaccines against Japanese encephalitis and tick-borne encephalitis (1.5 to 6 μg). Immune responses against XRX-001 were nevertheless strongly dose-dependent, suggesting that higher geometric mean titers and more durable immune responses might be achieved with higher doses of the purified antigen. This possibility should be investigated in phase 2 trials.

More than 90% of subjects in the high-dose vaccine group had antibody titers (measured as $PRNT_{50}$ titers) of 40 or more (Fig. 2), a titer exceeding the minimum protective level. The minimum protective level of neutralizing antibodies has been estimated in monkeys vaccinated with the 17D virus and challenged with yellow fever virus.[8] A different neutralization assay (the constant serum-varying virus assay) was used to

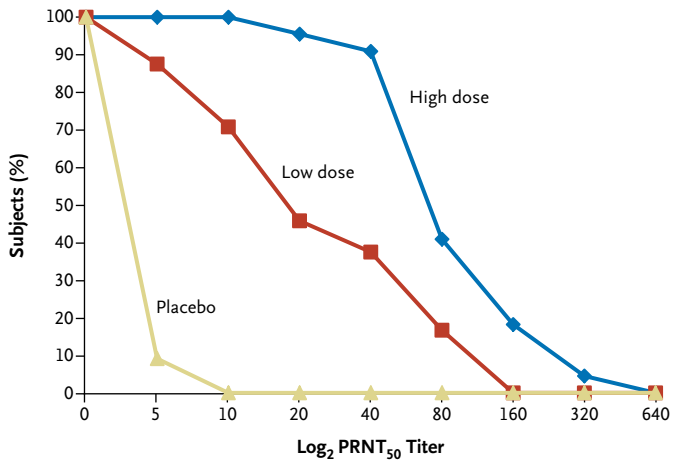

Figure 2. Cumulative Reverse Distribution of Plaque-Reduction Neutralization Test (PRNT) Titers of Neutralizing Antibody at Day 42, According to Study Group.

The $PRNT_{50}$ titer is the highest serum dilution inhibiting 50% or more of the plaques.

determine the immune correlate of protection in the studies in monkeys (i.e., a $\log_{10}$ neutralization index ≥0.7). This correlate has been used as a cutoff value in many clinical studies, including pivotal noninferiority trials of yellow fever vaccines,[9] whereas other studies have used a $PRNT_{50}$ of 10 as the minimal level of seroprotection.[5,6] The administration of antibody against XRX-001 and yellow fever 17D protected hamsters against a lethal challenge when the passive antibody titer was 20 or more.[10] Taken together, these results lead us to conclude that seroprotection in humans corresponds to a $PRNT_{50}$ titer of 10 to 20. Similarly, the World Health Organization[11] and the Food and Drug Administration[12] consider the immune correlate of protection for Japanese encephalitis vaccines to be a neutralizing antibody titer of 10 or more, measured as the $PRNT_{50}$ titer.

The safety and immunogenicity of XRX-001 are similar to those of Japanese and tick-borne encephalitis vaccines, although historical comparisons are compromised by differences in the neutralizing antibody assays used. The first inactivated Japanese encephalitis vaccine approved for use in the United States was JE-VAX, which required three injections — on days 0, 7, and 14 or 28 — for primary immunization. The rate of seropositivity and the geometric mean titer after three injections of JE-VAX[13] were similar to those elicited against yellow fever by two injections of XRX-001 in our study (Table 2 and Fig. 1 and 2), and the responses to XRX-001 were superior to responses after two injections of alum-adjuvanted tick-borne encephalitis vaccine.[14]

Travelers generally require short-term protection against yellow fever, but persistent antibodies are important for vaccination of indigenous populations in areas where yellow fever is endemic. The live 17D vaccine induces immune responses that are extremely durable and possibly lifelong. The duration of the neutralizing antibody response to XRX-001 will be determined after the 12-month follow-up period in our ongoing study. Vaccination with inactivated Japanese encephalitis has shown a decline in antibody titer over time, as was expected, but also showed persistence of protective antibody titers (≥10) in 83% of subjects for 12 months.[15]

A critical issue for the development of XRX-001 is the regulatory pathway to licensure. The efficacy will be estimated on the basis of the immune response, since field studies are not feasible. Although the proportion of subjects with seroprotection after administration of the XRX-001 vaccine may be equal to that after administration of the live 17D vaccine, the geometric mean titer and antibody durability with XRX-001 will most likely be inferior. Whether the potential benefit of improved safety will offset the lower antibody titers in regulatory decisions remains to be determined. Due to their low incidence,[2] a reduction in serious viscerotropic and neurotropic adverse events after administration of inactivated vaccine cannot be directly shown in clinical trials and will require postmarketing studies. The next steps necessary for the development of the XRX-001 vaccine include evaluation of the durability of the antibody response reported in the present trial and initiation of a double-blind, phase 2 clinical study.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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